Effects of Low Dose Radiation and Radiation Countermeasures on Infection by Spaceflight Analogue Cultured *Salmonella* Using 3-D Biomimetic Human Tissue Models

Jennifer Barrila[1], Sandhya Gangaraju[1], Laura Banken[1,2], Jiseon Yang[1], Richard R. Davis[1], Audrie A. Medina-Colorado[3], Ji Sun Park[4], Eleanor A. Blakely[5], Phillip Stafford[6], C. Mark Ott[7], and Cheryl A. Nickerson[1,2]

[1]Biodesign Center for Fundamental and Applied Microbiomics; Arizona State University, Tempe, AZ; [2]School of Life Sciences, Arizona State University, Tempe, AZ; [3]KBR, Houston, TX; [4]Enzychem Lifesciences, South Korea; [5]Lawrence Berkeley National Laboratory, Berkeley, CA; [6]Biodesign Center for Innovations Medicine, Tempe, AZ; [7]NASA Johnson Space Center, Houston, TX

Abstract

While both microgravity and radiation are major biological stressors associated with the spaceflight environment, their cumulative impact on host-pathogen interactions and infectious disease risks are rarely considered. This is critical to address, since the cumulative effects of these stressors during spaceflight may result in unexpected negative impacts on crew health and performance that neither condition alone would predict, thus limiting the ability to develop effective countermeasures. Previously, we showed that both spaceflight and spaceflight analogue culture increased the virulence and pathogenesis-related characteristics of the foodborne pathogen, *Salmonella* Typhimurium (*S.* Typhimurium), which is responsible for disqualification of food destined for the International Space Station and *Salmonella* spp. have been found aboard NASA spacecraft. Recently, we demonstrated that spaceflight-analogue culture of *S.* Typhimurium increased its ability to infect 3-D biomimetic human intestinal tissue models. In a separate study, we showed low dose radiation damaged our 3-D intestinal models. The primary objective of this proposal is to evaluate the possibility that low dose radiation will exacerbate the already increased bacterial pathogenicity of *S.* Typhimurium observed following spaceflight analogue culture. In addition, we will determine the impact of a radiation countermeasure to provide protection against both radiation and pathogen-induced tissue damage and inflammation.

Hypothesis: The already enhanced infection potential of spaceflight analogue cultured *S.* Typhimurium will be further exacerbated when used to infect host cells exposed to low dose radiation and this enhanced pathogenicity can be mitigated by a radioprotective compound.

Aims: 1. Characterize the impact of spaceflight-analogue culture on the ability of *S.* Typhimurium to infect 3-D biomimetic intestinal tissue models before and after exposure to low dose radiation. 2. Evaluate the ability of the radioprotective compound, EC-18, to protect 3-D intestinal models from low dose radiation, *S.* Typhimurium infection, and the cumulative impact of these stressors.

Significance: Current infectious disease risk assessments for spaceflight do not consider the potential for increased susceptibility to infection and disease resulting from exposure to low dose radiation, which is a critical consideration. This study will provide key evidence to determine if exposure to low dose radiation may be a factor in astronaut susceptibility to infection during long duration exploration missions and the impact of selected countermeasures to mitigate that risk to crew health. This study has been initiated and data collection is beginning.

Introduction

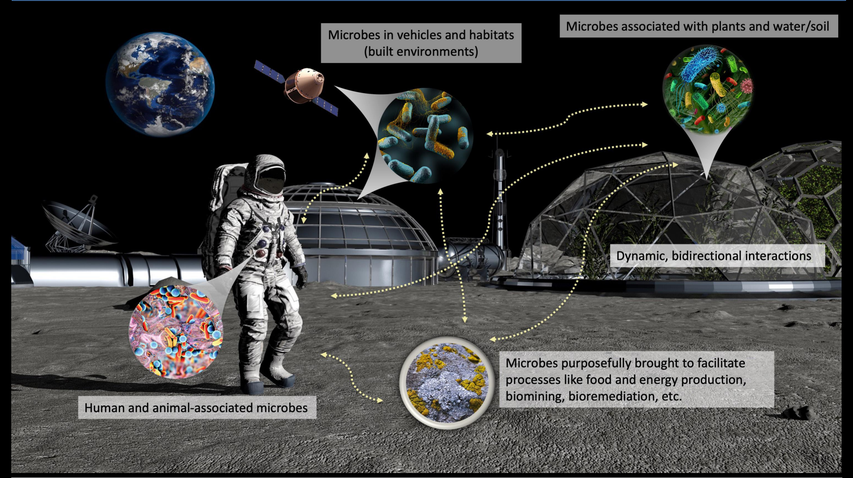


Fig. 1. Wherever humans travel, they carry with them trillions of microorganisms that can be either beneficial or harmful. It is critical to understand how stressors present in different space environments can alter the function of both human and microbial cells in order to anticipate and mitigate risks for crew health, safety, and mission success.

How do spaceflight stressors impact host-pathogen interactions and infectious disease risks?

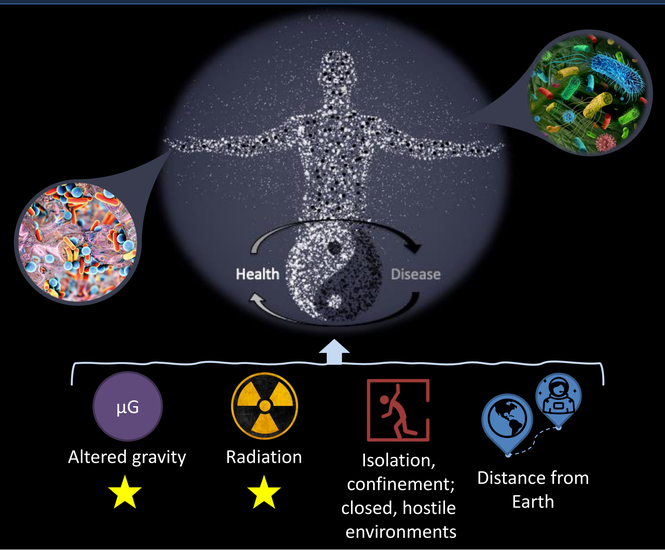
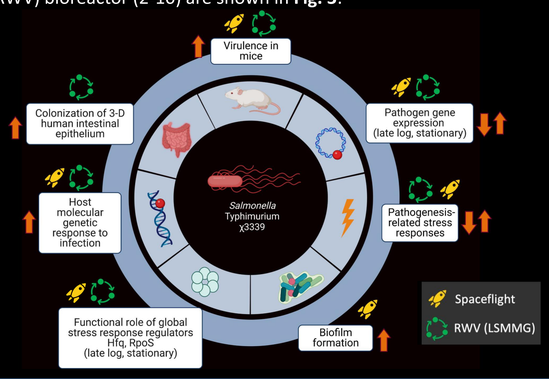


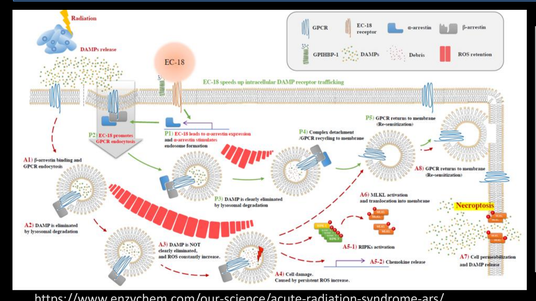
Fig. 2. Multiple stressors present during spaceflight – such as microgravity and radiation – have been shown to impact (or have the potential to impact) humans and/or microbes and the host-pathogen interactions that lead to infection and disease. Starred icons indicate key stressors being explored in the current project.

- Foodborne and waterborne pathogens represent major health risks for spaceflight missions in Low Earth Orbit (LEO) and deep space. This is important to consider since spaceflight has been associated with dysregulated immune function (1).
- Previous molecular genetic and functional studies with the enteric pathogen *Salmonella* Typhimurium in response to spaceflight and Low Shear Modeled Microgravity (LSMMG) culture in the Rotating Wall Vessel (RWV) bioreactor (2-10) are shown in **Fig. 3**:

Fig. 3. Spaceflight and LSMMG culture globally alter the virulence, gene expression and pathogenesis-related stress responses of *S.* Typhimurium. This figure briefly summarizes over two decades of *in vitro* and *in vivo* research findings for this enteric pathogen in these environments.



EC-18: a radioprotective and infection anti-inflammatory compound with potential spaceflight applications



https://www.enzychem.com/our-science/acute-radiation-syndrome-ars/

Fig. 4. EC-18 is an immune modulator that accelerates the inflammatory immune response by quickly removing the PAMP/DAMP-associated danger signals. Due to early termination of the immune response, EC-18 contributes to rapid resolution of inflammation and return to homeostasis. The FDA has granted Orphan Drug Designation to EC-18 for the treatment of Acute Radiation Syndrome (ARS).

Evaluating whether radiation exposure exacerbates infection by *Salmonella* and efficacy of a candidate countermeasure

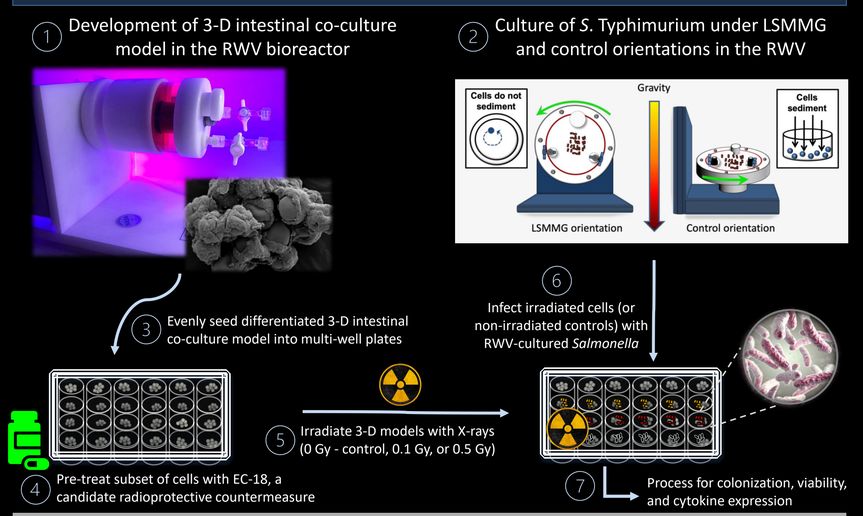


Fig. 5. Experimental overview. (1) 3-D co-culture models of human colonic epithelium containing HT-29 epithelial cells and PMA-differentiated U937 macrophages are grown in Slow Turning Lateral Vessel (STLV) bioreactors for 14 days as described (8, 10). **(2)** *S.* Typhimurium strain χ3339 is grown in the RWV for 24 hr under LSMMG and control conditions at 37 C in LB. Non-invasive control strain *E. coli* HB101 is grown shaking for 24 hr. **(3)** Models are harvested and evenly seeded into multi-well plates. (4) A subset of wells are pre-treated with EC-18, a candidate radioprotective countermeasure. Untreated controls are given 0.05% DMSO (vehicle). **(5)** 3-D models (treated and untreated) are irradiated with either 0, 0.1 or 0.5 Gy 320 kV X-rays (0.1 Gy/min) on a Precision X-ray, Inc. X-RAD 320 X-ray irradiator. **(6)** Bacteria are harvested and added to the 3-D co-culture model at a multiplicity of infection of 10. **(7)** Supernatants are collected and processed for lactate dehydrogenase assays (LDH/viability) and cytokine analyses. For colonization studies: at 30 minutes post-infection (adherence), samples are rinsed with Hank's Balanced Salt Solution (HBSS) in triplicate. A subset of the wells receive 0.1% sodium deoxycholate to lyse host cells and remaining wells receive media containing 50 µg/mL gentamicin. Lysed samples are serially diluted and plated. At 3 hours post-infection (h.p.i.), a subset of the samples are washed with HBSS, lysed in 0.1% sodium deoxycholate, serially diluted and plated to evaluate the percentage of bacteria that have invaded into the cells. Remaining cells receive media containing gentamicin at 10 µg/mL. Washes, serial dilution and plating steps are repeated at 24 h.p.i. for remaining wells for intracellular survival.

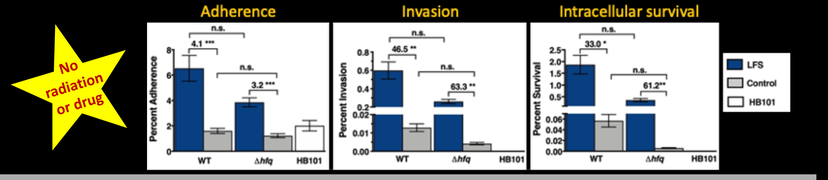


Fig. 6. In the absence of radiation and drug treatments, LSMMG culture enhances the adherence, invasion and intracellular survival of wild type and Δ*hfq S.* Typhimurium χ3339 in a 3-D human intestinal co-culture model (8). Infections were performed as shown in Fig. 5 (without radiation and drug treatments). Colony counts obtained from plating at each time point were normalized to the initial inoculum. Results are shown as mean ± standard deviation. Data were assessed for normality using the Shapiro-Wilk test and were analyzed using Kruskal–Wallis non–parametric ANOVA with Dunn's multiple comparisons. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Ongoing studies

Experimental conditions for the irradiation, drug treatments and infections have been tested and optimized using the 3-D co-culture model, EC-18 and *S.* Typhimurium. We are in the process of finalizing data collection – including colonization studies, viability, imaging and cytokine analyses.

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Acknowledgements

This work was supported by NASA Human Research Program grant 80NSSC21K1024.